AD-A253 252

ITATION PAGE

Form Approved
OMB No. 0704-0188

	T DATE	3. REPORT TYPE AND DATES COVERED ANNUAL 01 Dec 88 TO 30 Nov 89

4. TITLE AND SUBTITLE
NEW INVESTIGATOR AWARD

5. FUNDING NUMBERS
G- AFOSR-89-0192
PE- 61102F
PR- 2312
TA- A5
(2)

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...59

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10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

DTIC
ELECTE
JUL 22 1992
S A D

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release;
distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

Microbial communities were collected on artificial substrates from Spring Creek and Douglas Lake at different times of the year. Communities were exposed to copper under continuous flow conditions using the artificial substrata - microcosm procedure of Pratt and Bowers (1990.) Several endpoints were monitored and included both structural and functional measures. MATC's (maximum allowable toxicant concentrations) were calculated based on significant responses and community sensitivity during different seasons within the same ecosystem and between different ecosystems. Based on these results, the biotic factors which are important in influencing community response to toxic impacts were determined.

92-19269

14. SUBJECT TERMS

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
(U)	(U)	(U)	(U)

Annual
~~Final~~ Technical Report

SETAC/AFOSR New Investigator Program

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June 1991

INTRODUCTION

The goal of the research presented herein was to examine the effect of ecosystem variability on the process of ecological risk assessment. Many ecosystems are superficially similar but have differing species compositions and seasonal productivity maxima. Interspecies extrapolations for protecting ecosystems may be flawed if ecosystem differences are not accounted for. Understanding the interaction of toxic chemicals and ecosystem variability is fundamental to validating predictions of environmental saftey. If ecosystem responses to stress are highly variable then additional information regarding exposure patterns will be needed to regulate chemical stressors. If ecosystem responses to stress is less variable and less sensitive than the response of individual species, then valid risk assessments could be based on simpler data bases.

Preliminary examination of microbial communities in a moderately hard, fourth order stream located in central Pennsylvania (Spring Creek, Centre County) and a mesotrophic lake located in northern Michigan (Douglas Lake, Emmitt County) revealed differences with respect to protozoan species composition and microbial biomass. We also found marked seasonal fluctuations in protozoan species richness and microbial biomass within ecosystems (see Table 1). The research reported here was conducted to determine the effect of this within and between ecosystem differences on the sensitivity of microbial communities to toxic exposure.

Microbial communities were collected on artificial substrata from Spring Creek and Douglas Lake at different times of the year. Communities were exposed to copper under continuous flow conditions using the artificial substrata - microcosm procedure of Pratt and Bowers (1990). Several endpoints were monitored and included both structural and functional measures. MATC's (maximum allowable toxicant concentrations) were calculated based on significant responses and community sensitivity during different seasons within the same ecosystem and between different ecosystems were determined. Based on these results, we have attempted to determine the biotic factors which are important in influencing community response to toxic impacts.

METHODS

The effect of copper on naturally derived microbial communities collected from two different ecosystems was evaluated using a microcosm procedure described by Pratt and Bowers (1990). Seasonal effects on community sensitivity was analyzed by conducting experiments during different months. A total of 5 experiments were conducted from August 1989 to August 1990; two using communities from Spring Creek and three using communities from Douglas Lake. Data from a similar copper experiment conducted in November 1988 using Spring Creek communities is also included for comparative purposes.

Ecosystems

The two source ecosystems used in this study were Spring

Creek (Centre County, PA) and Douglas Lake (Emmitt County, MI). Spring Creek is a free-flowing second order stream of moderate hardness. The stream is relatively unimpacted by point and nonpoint source pollutants above the site where microbial communities were collected. Douglas Lake is small, relatively mesotrophic lake located in the northern tip of lower Michigan. It is moderately hard with few anthropogenic impacts.

Microbial Communities

Microbial communities from the two ecosystems were collected using polyurethane foam artificial substrata (Cairns et al. 1979; Pratt and Bowers 1990). Artificial substrata were suspended in the water column to become colonized by natural microbial communities, including bacteria, protista, and micrometazoa. Hundreds of species can be collected in this manner and easily transported back to the laboratory for manipulation. In lotic systems such as Spring Creek, colonization occurred rapidly and substrata were typically left for 5 to 7 days to accumulate organisms. Microbial colonization in lentic systems generally occurs slower, so substrata were left for 3 to 4 wks to ensure maximum species accumulation (Cairns et al. 1979).

Prior to the start of each new test, clean artificial substrata were placed in the selected ecosystem and colonized for the appropriate amount of time. In the Spring Creek tests, substrata were collected the morning of the test and returned to the laboratory. In the Douglas Lake tests, substrata were collected the day before the test and shipped overnight in an

insulated container to our laboratory at The Pennsylvania State University (preliminary tests have shown that substrata handled in this manner do not undergo any appreciable changes).

Tests were initiated by placing two colonized substrata (epicenters) into each of 15 to 18 microcosms containing dechlorinated tap water ammended with copper and then left for 7 days. Three additional substrata were analyzed immediately to determine protozoan species richness and composition, total protein, chlorophyll *a*, and alkaline phosphatase activity.

Toxicant

Microbial communities were exposed to copper under flow-through conditions using a proportional diluter described by Benoit et al. (1987). Triplicate microcosms were used for each concentration tested. Nominal concentrations in all tests were the same unless specified and were 0 (control), 10, 20, 40, 80, and 160 ug Cu/L as $CuSO_4$. Water samples were collected at the beginning and end of each test for copper analysis. Details of the microcosm testing procedure can be found in Pratt and Bowers (1990).

Endpoints

Several structural and functional parameters were measured to evaluate the sensitivity of each microbial community to copper. Dissolved oxygen was measured each afternoon just prior to lights out in each microcosm and production to respiration ratios were estimated based on the three point dissolved oxygen method of McConnell (1962) at the end of each experiment.

Exposed substrata were removed from each microcosm at the end of the tests and microbial communities collected by squeezing the contents into a sterile plastic beaker. Subsamples were then removed for protein, chlorophyll a, alkaline phosphatase activity, and protozoan species analysis. Specific details of these analyses can be found in Pratt and Bowers (1990). In addition, analyses for potassium, calcium, and magnesium were conducted in some of the tests.

Data Analysis

Microbial community responses were compared using analysis of variance (ANOVA, Sokal and Rohlf 1983). When treatment responses differed significantly from control (alpha = 0.05), multiple comparisons were made using Fisher's LSD (Sokal and Rohlf 1983). The maximum allowable toxicant concentration (MATC) for each significant response was calculated based on the geometric mean of the no-observable effect concentration (NOEC) and the lowest observable effect concentration (LOEC). In some cases, the LOEC occurred at the lowest concentration tested, and therefore, no MATC could be calculated. In addition, EC_{05}s and EC_{20}s (those concentrations corresponding to a 5% and 20% change in response relative to the mean control response) were inversely predicted from regression lines (Sokal and Rohlf 1983) for selected significant responses.

RESULTS

Source Communities

Protozoan species richness and composition of microbial

communities collected from Spring Creek and Douglas Lake are shown in Table 1. This data is from artificial substrata which were analyzed at the start of each test. Microbial communities from Spring Creek were used in three copper tests which were conducted during the late fall (Nov88), mid-winter (Feb90), and mid-spring (Apr90). Douglas Lake microbial communities were tested three times as well, twice during late summer (Aug89 and Aug90) and once during the late spring (May90).

Species richness ranged from 32.7 to 52.0 in Spring Creek and in Douglas Lake, ranged from 36.3 to 67.3. Communities in Douglas Lake tended to have a greater proportion of photosynthetic species than Spring Creek, but values overlapped between the two communities. Biomass estimates and alkaline phosphatase activities are shown in Table 2. Biomass tended to be lower in Douglas Lake communities and values were not consistant from year to year. Alkaline phosphatase activity was greater in the Douglas Lake communities, which is probably reflective of the lower phosphorus concentrations in Douglas Lake as compared to Spring Creek.

Microbial Responses

Realized copper concentrations for each microcosm experiment are shown in Table 3. These concentrations were used in the inverse predictions for EC05s and EC20s based on species richness (Table 4). Complete data for each test can be found in Appendix I. The range of sensitivities of the two communities to copper

were very similar and there was not a discernable seasonal pattern.

Maximum allowable toxicant concentrations were calculated based on structural and functional measures (Table 5). Species richness and total biomass were the most sensitive measures of copper stress, although most responses measured were sensitive to copper at or below 100 ug/L.

For nearly all tests, adverse effects were observed at or below the water quality criteria. Effect levels are all similar with the exception of the third experiment using MI microbial communities. The second and third MI experiments were conducted at Penn State University using a different dilution water, and this may have contributed to high variability in effect levels. The first MI experiment was done at Douglas Lake using Douglas Lake dilution water, and effect levels were in the range of the other experiments.

Effect levels are similar within classes of measures (species richness, community function) in all experiments. Effect levels for biomass measures are more variable among experiments within a site than other measures, and this may be due to seasonal differences among experiments. Results of experiments conducted using microbial communities derived from the same source ecosystem are similar, suggesting repeatability of results within an ecosystem. Interecosystem comparisons are less similar due to the second and third MI experiments. However, results both within and among regions are quite similar with effect levels

varying approximately an order of magnitude. This variability is similar to or less than that reported for single species acute tests of many compounds (Odum et al. 1979) using methods that are simple and standardized. These experiments indicate that our artifical substrata microcosm test system is repeatable when standard procedures are followed. Similar results were obtained by Taub and colleagues (1986, 1989) when testing the Standard Aquatic Microcosm among several laboratories, although the concentrations tested were much higher (lowest copper concentration 500 μg/L).

Differences among ecosystems in response to toxic dose are more problematic. For example, the speciation of copper in these experiments clearly varied due to different water hardness and pH, but results do not vary greatly even though free cupric ion is probably not similar among experiments. In contrast, the relative sensitivity of communities from differing ecosystems is not understood, although it is widely assumed to be similar for purposes of regulation.

Measurement variability

Variables measured as microcosm responses to toxicants differ in their precision. Some measures have significant variability with both biological and procedural sources. For example, the measurement of chlorophyll a varies because replicate communities vary and because the methods for concentrating cells, extracting the pigment, and measuring the extract introduce additional error. Other variables can be

measured with less error. For example, spectrophotometric determination of macronutrients such as calcium have low variability. Other measures may be discrete data, such as the enumeration of species, also associated with low variability, so some measures will be naturally less variable than others.

The effect of measurement variability, in a statistical sense, is a reduction in the power to detect differences among treatments. The importance of measurement variability in ecotoxicology is the error that it may introduce into conclusions drawn from experiments. Adequate understanding of the effect of measurement error on the potential to detect effects is important in experimental design and interpretation of results (Conquest 1983; Giesy and Allred 1985).

To assess the variability of measures from our artifical substrata microcosm experiments, the responses of control microcosms were summarized and the median coefficient of variation (CV, the ratio of the standard deviation to the mean) was determined. Using this estimate of the variability of a particular measure, the minimum detectable difference (MDD) was determined as the smallest percentage difference between control and treatment means that could be detected given the expected measurement variability and the experimental design (Sokal and Rohlf 1983). The assumed design was six treatments of three replicates each. Using an alpha of 0.05 (the probability of a Type I error) and beta of 0.2 (the probability of a Type II statistical error), MDDs were determined for several measured

variables (Table 5).

The CVs and MDDs reported in Table 6 are similar to the variability often observed in single species tests and are no greater than those predicted (Taub et al. 1989) as typical for ecosystem experiments. In fact, many measures have low variability and detection power is high, so that differences of only 20% in means between toxicant treated microcosms and control microcosms can reveal significant differences, assuming variance is similar among treatments. Expectations that microcosm replicability is poor and large variability would confound interpretation of results are unfounded.

CONCLUSIONS

Ecological assessment of toxic chemicals requires estimating effects on complex ecological structures. If the goal of environmental protection is to conserve ecological diversity and ensure the continued integrity of ecosystems, then laboratory ecosytems (microcosms) can provide a rapid and sensitive means of evaluating the adequacy of conclusions drawn from traditional hazard assessments. Microcosms containing diverse communities display the predicted symptoms of ecosystem disease (Schaeffer et al. 1988) in a manner that is both repeatable and sensitive to many stressors. However, microcosm experiments, like single species tests, are not globally sensitive to all stresses. Where microcosms lack appropriate target species for toxicants with specific modes of action, little effect can be detected.

Toxicant effects are the result of complex interactions between the toxicant, the available biota, and abiotic factors resulting in responses that are not predictable from single species tests. Microcosms provide an opportunity to test hypotheses of environmental safety and harm in a manner that is rapid, sensitive, repeatable, and capable of demonstrating unexpected, adverse ecological consequences of toxic materials.

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Table 1. Initial protozoan community richness and functional group composition on artificial substrata collected from Spring Creek, PA and Douglas Lake, MI. Values are mean (standard deviation).

Source	Date	Total	% B	% P	% N	% A	% S
Spring Creek	Nov88	52.0 (3.60)	75.6 (6.45)	7.73 (1.11)	9.55 (2.34)	5.22 (1.56)	1.90 (0.77)
Spring Creek	Feb90	32.7 (5.50)	84.4 (2.71)	10.7 (7.74)	2.90 (3.04)	2.02 (3.50)	0
Spring Creek	Apr90	44.7 (2.52)	82.0 (4.94)	5.32 (2.77)	9.71 (1.32)	2.23 (0.12)	0.71 (1.22)
Douglas Lake	Aug89	57.7 (2.08)	77.4 (2.12)	11.0 (3.01)	8.64 (1.43)	2.89 (0.99)	0
Douglas Lake	May90	36.3 (2.08)	64.0 (6.24)	22.1 (3.96)	12.9 (2.16)	0.90 (1.55)	0
Douglas Lake	Aug90	67.3 (2.31)	72.3 (9.18)	12.0 (4.76)	8.86 (3.76)	4.52 (4.04)	1.96 (0.77)

Table 2. Initial biomass estimates of microbial communities on artificial substrata collected from Spring Creek, PA and Douglas Lake, MI. Values are mean (standard deviation).

Source	Date	Protein (ug/L)	APA[a]	Chlorophyll *a* (ug/L)
Spring Creek	Nov88	129 (14.7)	277 (15.5)	537 (93.4)
Spring Creek	Feb90	692 (137)	468 (26.6)	1951 (131)
Spring Creek	Apr90	374 (107)	170 (74.8)	1463 (967)
Douglas Lake	Aug89	44.4 (11.5)	1102 (277)	90.0 (11.1)
Douglas Lake	May90	34.6 (7.87)	992 (183)	173 (24.9)
Douglas Lake	Aug90	21.5 (3.59)	1890 (314)	148 (23.3)

[a] nmole p-nitrophenol/mg protein/h

Table 3. Copper concentrations in flow-through laboratory microcosm experiments using either communities from Spring Creek (PA) or Douglas Lake (MI). Values are ug/L.

Treatment	Spring Creek			Douglas Lake		
	Nov88	Feb90	Apr90	Aug89	May90	Aug90
Control	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0
10 ug/L	9.9	20.0	9.1	9.6	12.1	13.5
20	19.9	23.3	18.2	13.9	24.2	18.5
40	40.0	50.7	36.5	27.4	48.5	36.0
80	90.0	106	79.0	55.3	90.3	70.0
160	205	212	165	122	154	131

* Not measured.

Table 4. Predicted EC_{05}'s and EC_{20}'s (95% confidence intervals) based on protozoan species richness. Values are in ug/L.

Ecosystem	Date	EC_{05}	EC_{20}
Spring Creek	May88	9.09 (6.42 - 11.5)	17.8 (13.4 - 22.3)
Spring Creek	Feb90	0.33 ---[a]	6.24 ---[a]
Spring Creek	May90	6.46 (2.59 - 8.65)	24.7 (14.6 - 36.2)
Douglas Lake	Aug89	9.30 (4.50 - 8.15)	16.5 (10.2 - 18.3)
Douglas Lake	Apr90	14.7 (8.46 - 15.8)	24.5 (16.6 - 28.6)
Douglas Lake	Aug90	27.8 (6.66 - 17.6)	43.4 (30.1 - 69.4)

Table 5. Maximum allowable toxicant concentrations calculated based on responses of protozoan communities from Spring Creek and Douglas Lake exposed to copper. Values are in ug/L.

Response	Spring Creek			Douglas Lake		
	Nov88	Feb90	May90	Aug89	Apr90	Aug90
Protozoan Species	19.9	20.0[b]	9.10[b]	18.9	66.2	95.8
% Bactivores	40	NS	NS	NS	NS	NS
% Producers	205	NS	NS	82.1	NS	NS
Protein	9.9	NS	NS	11.2	17.1	NS
Alk. Phos.	NS	NS	25.8	NS	17.1	NS
Chlorophyll *a*	40	73.3	53.9	82.1	12.1[b]	95.8
Calcium	90	-	53.9	38.9	NS	-
Magnesium	NS	-	53.9	NS	NS	-
Potassium	90	-	9.10[b]	NS	12.1[b]	-
Dissolved Oxygen	40	NS	25.8	18.9	NS	NS

[a] Not measured.

[b] These are the lowest observable effect concentration, since effects occurred at the lowest concentration tested.

Table 6. Estimates of variability of response variables in laboratory microcosm toxicity tests. Tables values are number of experiments (n), median coefficients of variation (CV), and minimum detectable distance as a percent of the control mean.

Variable	n	CV (%)	Minimum distance %
Structure variables			
Species richness	27	7.3	18.1
Total protein	28	17.6	43.8
Chlorophyll a	22	22.1	55.0
Calcium	16	11.4	28.3
Magnesium	16	13.3	33.1
Potassium	16	16.5	41.1
Function variables			
Dissolved oxygen	18	4.5	11.3
pH	15	2.2	5.5
Alkaline phosphatase activity	26	18.0	44.8

APPENDIX I

Table 1. Protozoan species richness and composition in microbial communities exposed to copper in laboratory flow-through microcosms for 7 d. Communities were collected from Spring Creek and the test conducted in April 1990. Values are mean (standard deviation).

Treatment	Total	% B	% P	% N	% A	% S	% R
Control	48.0 (2.64)	55.9 (3.78)	3.17 (0.61)	3.94 (1.60)	3.17 (0.61)	0	0
10 ug/L	42.3 (4.04)	54.8 (3.00)	2.32 (1.72)	4.09 (1.49)	4.09 (0.37)	0	0
20	38.7[a] (2.08)	52.7 (3.10)	2.94 (1.35)	4.93 (0.59)	3.94 (0.75)	0	0
40	40.3[a] (2.52)	51.7 (4.71)	1.90 (0.84)	6.03 (1.41)	4.78 (1.10)	0	0
80	34.0[a] (3.46)	59.1 (5.32)	0.60 (1.03)	5.60 (0.38)	2.11* (2.28)	0	0
160	25.3[a] (3.05)	60.2 (2.74)	1.47 (2.74)	5.47 (2.55)	0.68* (1.18)	0	0
p	0.0001	0.1113	0.3531	0.4035	0.0142	-	-

[a] Significantly different from controls at $\alpha = 0.05$.

Table 2. Biomass estimates of microbial communities exposed to copper in laboratory flow-through microcosms for 7 d. Communities were collected from Spring Creek and the test conducted in April 1990. Values are mean (standard deviation).

Treatment	Pro. (ug/ml)	Alk. Phosp.[a]	Chl a (ug/L)	Ca (mg/L)	Mg (mg/L)	K (mg/L)
Control	402 (124)	138 (12.7)	1848 (260)	735 (183)	79.9 (12.7)	6.16 (1.63)
10 ug/L	295 (1.73)	165 (44.2)	1950 (527)	479 (140)	54.0 (16.6)	2.52[b] (1.28)
20	368 (99.4)	160 (26.1)	2247 (96.8)	650 (64.0)	67.2 (4.03)	4.01[b] (0.80)
40	229 (48.5)	284 (76.1)	1809 (398)	759 (85.3)	78.2 (6.42)	3.26[b] (0.25)
80	317 (29.9)	220 (53.3)	1142[b] (56)	1017 (282)	97.3[a] (30.7)	4.35 (1.13)
160	311 (59.6)	180 (45.6)	949[b] (92.7)	521 (51.2)	61.7 (4.77)	2.86[b] (0.29)
p	0.1476	0.0286	0.0011	0.0140	0.0581	0.0112

[a] nmole p-nitrophenol/mg protein/hr

[b] Significantly different from controls at $\alpha = 0.05$.

Table 3. Protozoan species richness and composition in microbial communities exposed to copper in laboratory microcosms for seven days. Communities were collected from Douglas Lake and the test conducted in May 1990. Values are mean (standard deviation).

Treatment	Total	% B	% P	% N	% A	% S	% R
Control	51.3 (6.66)	73.0 (8.58)	14.9 (3.05)	9.74 (3.29)	2.56 (2.26)	0	0
10 ug/L	47.7 (1.15)	75.5 (1.66)	11.9 (1.13)	10.5 (2.41)	2.09 (2.11)	0	0
20	45.3 (1.15)	75.0 (1.66)	14.7 (1.53)	9.55 (1.19)	0.71 (1.24)	0	0
40	45.7 (3.78)	77.2 (10.9)	16.0 (2.51)	5.86 (3.52)	0.61 (0.11)	0	0
80	17.7[a] (3.78)	84.5 (17.8)	11.0 (4.19)	5.25 (4.71)	0	0	0
160	11.3[a] (0.58)	79.4 (9.84)	18.2 (9.86)	2.78 (4.80)	0	0	0
p	0.0001	0.3081	0.4493	0.0925	0.1339	-	-

[a] Significantly different from control at $\alpha = 0.05$

Table 4. Biomass estimates of microbial communities exposed to copper in laboratory flow-through microcosms for 7 d. Communities were collected from Douglas Lake and the test conducted in May 1990. Values are mean (standard deviation).

Treatment	Pro. (ug/ml)	Alk. Phosp.[a]	Chl a (ug/L)	Ca (mg/L)	Mg (mg/L)	K (mg/L)
Control	33.6 (9.08)	229 (47.8)	379 (44.7)	39.6 (1.55)	20.8 (0.81)	1.04 (0.12)
10 ug/L	29.4 (7.66)	255 (49.6)	210[b] (34.7)	38.7 (20.4)	20.4 (0.25)	0.50[b] (0.14)
20	19.6[b] (9.41)	367[b] (115)	155[b] (55.5)	40.4 (3.60)	21.2 (1.21)	0.46[b] (0.06)
40	17.6[b] (1.35)	408[b] (30.0)	235[b] (47.4)	39.4 (1.95)	20.4 (0.26)	0.46[b] (0.03)
80	8.80[b] (2.94)	404[b] (93.9)	76.7[b] (4.71)	40.6 (4 71)	20.9 (1.73)	0.40[b] (0.02)
160	6.67[b] (1.55)	495[b] (13.6)	32.0[b] (20.7)	37.9[b] (0.75)	20.5 (0.00)	0.42[b] (0.11)
p	0.0012	0.0022	0.0001	0.8124	0.8743	0.0001

[a] nmole p-nitrophenol/mg protein/hr

[b] significantly different from control at $\alpha = 0.05$

Table 5. Protozoan species richness and composition in microbial communities exposed to copper in laboratory microcosms for seven days. Communities were collected from Douglas Lake and the test conducted in August 1989. Values are mean (standard deviation).

Treatment	Total	% B	% P	% N	% A	% S	% R
Control	73.0 (1.73)	76.7 (4.05)	11.3 (0.64)	9.36 (1.52)	2.39 (0.93)	0	1.36 (1.38)
10 ug/L	72.3 (7.02)	79.9 (5.48)	9.72 (0.97)	8.78 (3.16)	1.89 (1.01)	0	0
20	69.7 (12.2)	79.0 (4.24)	10.5 (2.60)	9.06 (0.59)	1.48 (0.24)	0	0
40	25.3[a] (5.51)	79.9 (7.46)	9.83 (4.59)	8.14 (1.78)	1.06 (1.87)	0	1.08 (1.87)
80	47.7[a] (10.9)	83.1 (8.75)	8.85 (2.15)	8.25 (2.91)	0	0	0
160	20.7[a] (8.96)	67.2 (14.2)	27.6[a] (14.4)	5.89 (5.72)	0.96 (1.64)	0	0

[a] Significantly different from controls.

Table 6. Biomass estimates of microbial communities exposed to copper in laboratory flow-through microcosms for 7 d. Communities were collected from Douglas Lake and the test conducted in August 1989. Values are mean (standard deviation).

Treatment	Pro. (ug/ml)	Alk. Phosp.[a]	Chl a (ug/L)	Ca (mg/L)	Mg (mg/L)	K (mg/L)	DO (mg/L)
Control	14.5 (3.39)	1371 (190)	132 (5.03)	55.0 (7.97)	19.1 (0.94)	4.87 (0.57)	8.53 (0.11)
10 ug/L	16.9 (1.78)	1208 (247)	129 (7.09)	46.1 (3.73)	16.1 (2.65)	3.90 (0.78)	8.38 (0.08)
20	19.8[b] (2.28)	1144 (140)	142 (37.1)	56.3 (5.05)	16.8 (1.30)	4.33 (2.12)	8.37 (0.20)
40	20.3[b] (1.73)	1148 (137)	146 (49.6)	51.7 (10.1)	16.1 (2.33)	2.70[b] (0.26)	8.40[b] (0.13)
80	18.3[b] (0.25)	1198 (113)	85.0 (21.0)	46.8 (3.16)	15.1 (1.58)	2.73[b] (0.35)	7.82[b] (0.08)
160	13.2 (0.42)	1479 (310)	54.0[b] (13.6)	47.1 (2.46)	15.1 (1.14)	2.77[b] (0.45)	7.88[b] (0.10)
p	0.0041	0.3179	0.0069	0.2268	0.1271	0.0123	0.0001

[a] nmole p-nitrophenol/mg protein/hr

[b] significantly different from control at $\alpha = 0.05$

Table 7. Protozoan species richness and composition in microbial communities exposed to copper in laboratory microcosms for seven days. Communities were collected from Douglas Lake and the test conducted in August 1990. Values are mean (standard deviation).

Treatment	Total	% B	% P	% N	% A	% S
Control	58.0 (6.56)	72.4 (0.79)	14.4 (1.25)	11.5 (0.75)	1.66 (1.55)	0
10 ug/L	57.3 (5.77)	75.2 (4.20)	14.1 (2.20)	8.35 (2.56)	2.27 (0.56)	0
20	50.7 (5.77)	75.5 (5.74)	14.1 (3.73)	7.83 (1.27)	1.99 (1.85)	0.61 (1.06)
40	48.7 (1.15)	79.5[a] (5.63)	10.2[a] (1.85)	9.57 (3.14)	0.70 (1.20)	0
80	49.3 (8.14)	81.2[a] (2.93)	7.43[a] (0.15)	8.65 (1.96)	2.06 (0.37)	0
160	31.7[a] (4.16)	77.6 (7.65)	10.6[a] (1.85)	10.5 (1.28)	2.18 (1.94)	0
p	0.0055	0.0540	0.0079	0.2623	0.7465	0.7465

[a] Significantly different from controls at alpha = 0.05.

Table 8. Biomass estimates of microbial communities exposed to copper in laboratory flow-through microcosms for 7 d. Communities were collected from Douglas Lake and the test conducted in August 1990. Values are mean (standard deviation).

Treatment	Pro. (ug/ml)	Alk. Phosp.[a]	Chl a (ug/L)	DO (mg/L)
Control	16.9 (5.63)	1510 (120)	178 (48.7)	10.8 (0.30)
10 ug/L	14.9 (3.43)	1365 (280)	147 (17.8)	10.8 (0.25)
20	11.9 (4.17)	1500 (234)	123 (3.60)	11.0 (0.15)
40	20.0 (4.19)	1265 (347)	162 (34.1)	10.7 (0.15)
80	14.8 (6.10)	1505 (322)	126 (38.5)	10.9 (0.15)
160	8.93 (3.48)	2260 (144)	75.3[a] (12.3)	10.8 (0.20)
p	0.1398	0.3179	0.0241	0.5644

[a] nmole p-nitrophenol/mg protein/hr

[b] Significantly different from controls at alpha = 0.05.

Table 9. Protozoan species richness and composition in microbial communities exposed to copper in laboratory microcosms for seven days. Communities were collected from Spring Creek and the test conducted in November 1988. Values are mean (standard deviation).

Treatment	Total	% B	% P
Control	41.0 (2.64)	69.2 (4.87)	16.3 (2.10)
10 ug/L	38.7 (4.61)	71.5 (8.95)	21.8 (3.51)
20	28.7[a] (2.08)	72.1 (5.14)	15.1 (0.97)
40	29.71[a] (2.51)	82.3[a] (12.7)	10.0 (3.05)
80	17.0[a] (1.00)	90.1[a] (9.29)	9.93 (3.86)
160	10.3[a] (0.58)	90.6[a] (1.15)	6.37[a] (5.53)
p	0.0001	0.0001	0.0007

[a] Significantly different from controls at alpha = 0.05.

Table 10. Biomass estimates of microbial communities exposed to copper in laboratory flow-through microcosms for 7 d. Communities were collected from Spring Creek and the test conducted in November 1988. Values are mean (standard deviation).

Treatment	Pro. (ug/ml)	Alk. Phosp.[a]	Chl a (ug/L)	Ca (mg/L)	Mg (mg/L)	K (mg/L)	DO (mg/L)
Control	253 (38.2)	190 (36.0)	552 (264)	102 (9.91)	23.3 (0.59)	7.00 (1.27)	15.7 (0.81)
10 ug/L	331[a] (6.43)	153 (15.3)	731 (164)	110 (15.6)	23.3 (0.36)	8.40 (0.40)	16.3 (0.98)
20	281 (17.4)	150 (2.64)	321 (88.3)	76.0 (27.5)	19.9 (3.69)	6.73 (0.84)	15.0 (0.35)
40	252 (11.8)	147 (5.80)	185[b] (42.0)	79.4 (12.6)	21.6 (0.25)	5.90 (0.15)	14.4 (0.84)
80	154[b] (20.5)	170 (36.0)	104[b] (37.0)	44.1[b] (1.00)	21.3 (1.07)	3.67[b] (0.67)	12.7[b] (0.65)
160	44.5[b] (0.71)	145 (35.3)	45.5[b] (7.78)	38.5[b] (2.12)	20.6 (0.99)	2.75[b] (1.20)	11.1[b] (0.20
p	0.0001	0.2302	0.0007	0.0008	0.1730	0.0001	0.0001

[a] nmole p-nitrophenol/mg protein/hr

[b] Significantly different from controls at alpha = 0.05.

Table 11. Protozoan species richness and composition in microbial communities exposed to copper in laboratory microcosms for seven days. Communities were collected from Spring Creek and the test conducted in February 1990. Values are mean (standard deviation).

Treatment	Total	% B	% P	% N	% A	% S
Control	35.3 (2.08)	80.9 (7.65)	8.54 (2.69)	6.63 (1.57)	2.81 (2.70)	2.81 (2.70)
10 ug/L	27.3[a] (3.21)	78.8 (10.4)	7.32 (0.88)	6.10 (4.17)	7.49 (2.10)	0.00
20	29.0[a] (0.00)	84.3 (12.9)	2.30 (3.98)	9.93 (2.89)	3.30 (3.23)	0.00
40	28.0[a] (3.00)	85.7 (6.19)	3.47 (3.24)	9.60 (2.88)	2.51 (3.23)	0.00
80	28.0[a] (2.64)	87.4 (20.8)	5.94 (2.04)	4.71 (4.12)	2.15 (3.73)	0.89 (1.55)
p	0.01470	0.6334	0.0976	0.1614	0.3347	0.1315

[a] Significantly different from controls at alpha = 0.05.

Table 12. Biomass estimates of microbial communities exposed to copper in laboratory flow-through microcosms for 7 d. Communities were collected from Spring Creek and the test conducted in February 1990. Values are mean (standard deviation).

Treatment	Pro. (ug/ml)	Alk. Phosp.[a]	Chl a (ug/L)	Ca (mg/L)	Mg (mg/L)	K (mg/L)	DO (mg/L)
Control	238.0 (26.0)	1806 (302)	5028 (1000)	DATA NOT COLLECTED			11.1 (0.40)
10 ug/L	252.5 (39.0)	1749 (141)	3946 (810)				11.8 (0.50)
20	246.7 (20.0)	1291 (65)	4546 (240)				11.1 (0.50)
80	214.8 (25.0)	1521 (113)	3456 (1100)				10.0[b] (0.30)
160	201.6 (26.0)	1782 (348)	1876[b] (560)				9.43[b] (0.30)

[a] nmole p-nitrophenol/mg protein/hr

[b] Significantly different from controls at alpha = 0.05.